

The future of molecular diagnostics for cancer: a 'personalised' perspective

The pharmaceutical industry is still struggling to cure cancer despite pouring enormous resources into the search for new treatments. We take a look at the some of the current technologies for the discovery and delivery of molecular diagnostic, prognostic and predictive tests and speculate on where this area is heading with regard to advanced technologies and likely future requirements.

It appears to be general practice now to use the term 'diagnostic' to cover three separate categories of test: diagnostic, prognostic and predictive. In so far as these are medical tests providing information about an individual, they can justifiably be considered together. Diagnostic, prognostic and predictive tests each contribute something different to our understanding, the sum of the parts helping to build a more complete picture of the patient's status. To remind ourselves:

- Diagnostic tests seek to reveal something characteristic or indicative of a disease.
- Prognostic tests provide information as to the likely future course and outcome of a disease.
- Predictive tests provide information on the likely response of a patient to a drug or therapy.

It is worth mentioning that current FDA practice seems to use the term 'diagnostic' generically in relation to testing, to cover all three categories.

Diagnosics

The current paradigm of cancer biology has it that tumours evolve through the accumulation of somatic mutations, which disengage cells from normal behaviour patterns, simultaneously confer-

ring growth advantage. The aim of the diagnostic is therefore to seek out direct or indirect evidence for the presence of that abnormal growth, preferably earlier rather than later in its journey from the benign to the malignant state.

Ideally such evidence should be collected with minimal discomfort (ie, in the least invasive manner) to the patient, hence the intense interest in tests that detect proteins or nucleic acids arising from the primary tumour site and found in the blood or in body fluids such as urine. But it is not clear at what stage in the development of a primary solid tumour such biomarkers will become detectable in these fluids. Although a tumour may not yet be invasive and therefore by definition not yet a cancer, once it has grown beyond a critical diameter it is invested with its own, new microvasculature, into which tumour cell components can be shed. Therefore it is likely that trace amounts of tumour protein and nucleic acids are present in the general circulation even at an early stage in its development. Of course detecting these molecules pre-supposes that they bear some characteristic that differentiates them from those associated with normal cells, and identifying 'tumour-specific' proteins or other classes of molecule has been the most challenging aspect of the search for circulating or

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fluid diagnostic markers. The issues therefore remain fundamentally unchanged; those are the issues of specificity and sensitivity. What are we measuring and how little of it can we detect?

While immunodiagnostic approaches continue to represent the largest proportion of FDA-approved cancer diagnostics, rapid developments in the use of mass spectroscopy have brought new power to the search for rare molecules or fragments of molecules in fluids. However; this technology suffers from questions around reproducibility and standardisation.

Next generation sequencing (NGS), owing to its massively ‘parallel’ nature, allows the simultaneous sequencing of many millions of individual DNA molecules. This provides scientists and clinicians with both exquisite sensitivity and specificity for the detection of abnormal DNA in solid tissues and in body fluids. To this end, several large pharma companies are investigating the feasibility of using NGS to detect somatic mutations in genes such as *TP53*, *KRAS* and *EGFR* in DNA in the circulation, as surrogate markers for the presence of a tumour. In addition, the sheer capacity of this technique allows multiplexing, so that many gene hotspots can be sequenced simultaneously. While NGS is economically and practically unsuited to routine application as a strictly ‘diagnostic’ tool, it is now in use in at least one centre in the UK for the routine screening for mutations in the *BRCA 1* and *2* genes in breast/ovarian cancer family members.

The standardisation and miniaturisation of technologies such as mass spectroscopy and next generation sequencing will probably make these the technologies of choice for the identification in the first instance, and then routine detection of, diagnostic markers in fluids. Indeed, through miniaturisation, these could ultimately become point-of-care technologies.

Prognostics

The value of more robust and sensitive ‘diagnostic’ tests, able to detect pre-malignant lesions or cancers at increasingly early stage is clear. Yet it has been argued that there is currently insufficient resource being given to early detection by, for example, imaging at the expense of new targeted drug development, ie treatment (more on this later). Between diagnostic and predictive tests, sit prognostic tests, the value of which is slightly less clear. Once the presence of a tumour is diagnosed and the disease is staged using standard clinico-pathological criteria, the oncologist or surgeon will have a reasonable idea of the prognosis without

resorting to molecular tests. How important this information is to the patient depends very much on the individual.

Nevertheless there is an argument for molecular prognostic tests in cancer, where routine staging fails to provide clear guidance as to the nature or intensity of any further treatment. The OncotypeDx™ test, based upon the analysis of expression of 21 genes, quantifies the likelihood of disease recurrence in women with early-stage hormone receptor-positive breast cancer, and as such is prognostic. This clinical situation is not uncommon, where it is unclear whether to offer chemotherapy, with its unpleasant side-effects. The evidence is that clinical practice in this situation varies significantly internationally, and nationally in the UK. A similar problem occurs with Stage 2b colorectal cancer, where there is often uncertainty as to whether to treat with adjuvant chemotherapy following surgery, and multiplexed molecular tests are under development to address this.

One would think that the availability of objective tests that offer an individualised risk assessment in these situations would be attractive. Unfortunately such tests remain problematic for the UK and many other national health services, primarily due to their cost. Against a somewhat bleak economic background, there is also an inherent conservatism about the need for rock-solid evidence bases, and perhaps a degree of complacency concerning the adequacy of current clinico-pathological evaluation.

Interestingly, the OncotypeDx test, in addition to providing an individualised probability of disease recurrence, has also been shown to be useful in assessing the likely benefit of chemotherapy, which makes it both ‘prognostic’ and ‘predictive’ (see below).

Predictives

Perhaps the most exciting advances in diagnostics are those around ‘predictives’: finding biological markers that predict likely response to a drug or treatment. There are a number of drivers contributing to these advances.

Several years ago the FDA and EU, in separate reports, highlighted the need for better “product evaluation tools” in the drug development process, tools which would produce safer and more effective drugs, and in turn lead to improved patient outcome^{1,2}. Behind this lay two uncomfortable facts: the first was that the cost to pharmas of taking a single, new anti-cancer agents from discovery to licensing had grown to unsupportable levels (currently estimated to be in excess of \$1 billion);

Table 1: Examples of types of changes occurring in genes or their protein products associated with tumours, which may predict for response to anti-cancer drugs

TYPE OF BIOCHEMICAL CHANGE ASSOCIATED WITH TUMOUR	GENE OR PROTEIN BIOMARKER	MAY PREDICT RESPONSE TO
SOMATIC MUTATIONS	<i>KRAS</i>	cetuximab; panitumumab; gefitinib; erlotinib
	<i>BRAF</i>	cetuximab; panitumumab; gefitinib; erlotinib
	<i>PIK3CA</i>	cetuximab; panitumumab; lapatinib; trastuzumab
	<i>EGFR</i>	gefitinib; erlotinib
INHERITED MUTATIONS	<i>BRCA1/2</i>	olaparib
POLYMORPHISMS	<i>UGT1A1</i>	irinotecan
	<i>CYP450</i>	tamoxifen
	<i>Fc RII/III</i>	trastuzumab; rituximab; erbitux
GENE AMPLIFICATION OR COPY NUMBER VARIATION	<i>HER2</i>	trastuzumab
	<i>EGFR</i>	gefitinib; erlotinib
	<i>Top2A</i>	anthracycline chemotherapy
GENE EXPRESSION (21 GENE MULTIPLEXED)	OncotypeDx™	chemotherapy
PROTEIN OVER-EXPRESSION	EGFR	cetuximab
	HER2	trastuzumab
	ERCC1	platinum compounds
	B-tubulin	Taxanes
PROTEIN LOSS; MUTATIONS, COPY NUMBER VARIATION	PTEN	trastuzumab; hormone therapy, chemotherapy

and the second (contributing to the first), was that the attrition rate for taking these drugs to licensing (most failing at the Phase II/III transition) was unacceptably high. For example, between 1990 and 2006 there were a total of 920 candidate therapeutics; of which 45% of these had been completed by 2007, but only 32 had been approved by the FDA. This equates to a startlingly low overall success rate of 8%³.

The FDA suggested that new types of “tools” were required to deal with this unacceptable situation. These fell into two categories:

- New molecular biomarkers, to be developed and incorporated into diagnostic assays.
- Proteomic and genomic signatures for diagnos-

tic, prognostic or predictive purposes, and for patient stratification and salvage of failed therapeutic agents.

The modern paradigm therefore became that the predictive (or ‘companion diagnostic’ in some instances) would evolve as part of a rational drug discovery programme, and that the predictive would accompany the drug through its developmental arc; from discovery, through clinical trials where it would be used to stratify patients, finally to companion test on the label of the newly-licensed therapeutic. Current examples of this are the HER2 immunohistochemistry with reflex FISH test that accompanies prescribing of Herceptin™ (trastuzumab) for advanced breast

and gastric cancers, and the *KRAS* mutation test, which is on the label of the anti-EGFR therapeutic antibody cetuximab (Erbix™) for advanced colorectal cancer.

Both of these are examples of a rational approach to the development of a predictive. However, not all current predictives are the product of such an approach; many have been discovered after the fact, as a result of the enormous power of modern genetic analysis techniques (Table 1). Interestingly, molecular (DNA/RNA-based) technology was adopted by the infectious diseases drug development sector long before the oncology sector picked up on it, and to that extent the oncology field has been playing catch-up.

Another important driver in the rush to discover new predictive biomarkers has been the (ethically commendable) desire to extract as much information as possible from the valuable patient samples that arise as part of clinical investigation or from surgical resection. Hospital pathology departments have accumulated many decades of archival material preserved with formalin. Until recently this material was only of utility for standard histopathology tests, which included immunohistochemistry and, more recently, fluorescence *in situ* hybridisation (FISH). However, the development of techniques for extracting and analysing DNA, RNA and proteins from tissue originally removed from a patient several decades ago has produced a wealth of new information about the relationship between the genetic make-up of an individual, their disease prognosis and likely response to drugs (pharmacogenomics). Therefore, with the benefit of retrospective analysis of patients whose disease was diagnosed and treated in the past, we can now make more informed and accurate prediction about the prognosis and likely response to treatment of a newly-diagnosed patient.

While the costs of such global analysis of a patient's DNA are currently too great for the resources of the UK's National Health Service, the global landscape is changing rapidly. In the US, the Dana-Farber and Memorial Sloan Kettering Cancer Centres are introducing routine profiling of a comprehensive set of 1,200 somatic mutations and key polymorphisms in 150 cancer-associated genes using mass-spectrometry. Cancer Research UK, in collaboration with Pfizer and AstraZeneca, has initiated a 'Stratified Medicine Programme' that will evaluate the feasibility of prospectively analysing key sets of somatic mutations and polymorphisms with known predictive value in approximately 9,000 patients over two years. In parallel, the UK Technology Strategy Board is

offering competitive awards to academic and commercial organisations to collaborate on the development of new technology for 'tumour profiling'. It is clear that NGS is likely to play a significant part in these developments, based on its unparalleled ability to scan the entire genome in an unbiased manner, at the same time looking at each nucleotide multiple times.

The future is imaging

For all the power and beauty of modern molecular technology, the future of diagnostics (diagnostics, prognostics and predictives) must lay in imaging. Imaging will be used to diagnose the presence of a tumour, to characterise that tumour and to monitor its response to treatment. In terms of anatomical information computerised tomography (CT) and magnetic resonance imaging (MRI) are the most accurate imaging modalities. However, these techniques often lack the ability to distinguish between tumour and normal tissues. 'Molecular' imaging using positron emission tomography (PET) with ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) allows clearer visualisation of tumours and their metabolic activity, based upon their enhanced glucose metabolism. Other PET tracers that can be used to track characteristics such as proliferation, hypoxia and expression of key receptors such as HER2 and EGFR, are being increasingly used in evaluation of tumours, and may have predictive power for response to treatment. Recent studies have suggested the added benefits of integrated PET-CT, which will combine functional and anatomical imaging.

Final thoughts

In a recent article⁴ Robert Landreth discussed the question of why pharma are struggling to cure cancer, despite pouring resources into the search for new treatments. Some drugs that make it through to licensing do show benefit, but more often than not for a small proportion, a subset of patients with a particular type of cancer. In the case of many so-called targeted drugs there is also now a 'companion diagnostic', which more often than not assists in excluding patients from treatment. As an eminent oncologist said to me the other day: "The problem is that with all these tests, soon I'll have nothing I can offer my patients." There is a hard truth to this slightly cynical remark. Garth Anderson of Roswell Park Cancer Institute being interviewed in the same article points out that with new technology we will reveal more and more mutations and other genetic changes. Furthermore this is a dynamic situation; changes are occurring all the time. Many of these

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References

- 1 FDA Critical Path Opportunities Report March 2006.
- 2 European Union Seventh Framework Programme (2007-2013).
- 3 Reichert, JM and Wenger, JB (2008). Development trends for new cancer therapeutics and vaccines. *Drug Discovery Today* 13:30.
- 4 <http://blogs.forbes.com/robertlangreth/2010/08/06/why-pfizer-cant-cure-cancer/>.

will exclude patients from new drugs. He argues that the paradigm of targeted therapy is inherently flawed and it may be time to go back to the beginning; more resource should be invested into early detection by imaging and improved surgical techniques. Perhaps it is time to shift emphasis from treatment to true diagnostics. The technology is available, with NGS and mass-spec leading the way, however diagnostics development needs further incentivising at the academic and research level. Pharmas are not about to drop research on targeted drugs in favour of diagnostic tests, I suspect the economics do not work, and drugs will always be needed in any case. Perhaps a change in perspective is called for. **DDW**

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